CONT 02

[USSN - 08/488,164]

Q122E, T123G, G126L, R127I and E129S.

Please add the following new claims:

74. The DNA molecule of claim 29 wherein the amino acid sequence of the antagonist comprises alpha helices corresponding to the alpha helices at about residues 4-33, 66-80, 108-127 and 150-179 of bovine growth hormone.

REMARKS

1. The Definiteness Issue

It is our understanding that in late October, Group Directors Dall and Kittle signed a policy memorandum instructing their examiners to refrain from making or maintaining 112/2 indefiniteness rejections on the basis of the alleged indefiniteness of "percentage identity" language for failure to adequately disclose the alignment algorithm, alignment parameters, or manner of calculating the percentage identity for a given alignment. Hence we expect that the rejection stated in §§7-8 of the last office action will be withdrawn in view of the aforementioned policy memorandum.

However, for the sake of the record, we will briefly address the merits of the rejection.

In our last amendment, we stated

> At page 12, lines 6-10, Applicants make reference to the use of the "Micro-Genie" program for secondary structure prediction. Any person skilled in the art would have recognized that this was a general sequence analysis program, and, in the absence of instructions to the contrary, would have used if for any other sequence analysis, including sequence alignment, required by the specification and claims.
>
> The "Micro-Genie" program (see User Manual December 1998, section 8.3, copy enclosed) offered two methods for sequence alignment: alignment by identity (option I) and alignment by similarity (option S).

> In option I, the sequences were aligned, with gapping allowed, to maximize the quantity S=M-G-N, where M is the number of matches, G is the number of gaps, and N is the number of residues contained in the gaps. (This is equivalent to a scoring matrix with +1 for each match and 0 for each mismatch, with a gap open penalty of -2 for the first null and a gap extension penalty of -1 for each additional null.)
>
> In option S, the sequence are aligned by another algorithm "which is described by M. Dayhoff et al. in the Atlas of Protein Structure". While the exact literature citation is not given, a review of the Atlas for the period preceding the first U.S. filing reveals several Dayhoff publications in the Atlas, the most recent being Dayhoff, et al., "Model of Evolutionary Change in Proteins", 5 (Suppl:3):345-52 (1978) and "Matrices for Detecting Distant Relationships", 5 (Suppl:3):353-58 (1978) (copies enclosed).[1]

The Examiner does not appear to question that, if the "Micro-Genie" program were explicitly indicated as the means for sequence alignment, that the alignment would then be unambiguous. Rather, the Examiner argues that "the instant specification never discloses that the 'Micro-Genie' program could be used for this purpose or should be used for this purpose over any other algorithm which was available to one of ordinary skill in the art. There is no implicit or explicit suggestion in the specification that this program was intended for the calculation of percent identity".

We disagree. By explicitly teaching the use of "Micro-Genie" to analyze the amino acid sequence of a GH mutant --albeit

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[1] While the user manual does not point this out, Merrifield, "MicroGenie:Homology Searches", Chap. 19 in Meth. Mol. Biol., vol. 24:Computer Analysis of Sequence Data, Part I (Griffin and Griffin, eds., 1994) (copy enclosed), at page 249, section 3.2.4, step 7 state that alignment by similarity is the default.

to predict the secondary structure-- applicants implicitly taught (absent any express teaching to the contrary) its use for any amino acid sequence analysis, including sequence alignment. A person skilled in the art would have been well aware that "Micro-Genie" had this capability.

2. Enablement Issue

2.1. The Examiner urges that "one of skill in the art could not delete nearly half of the growth hormone protein and expect it to bind the receptor in order for it to function as a GH receptor antagonist". Simultaneously, the examiner concedes that the 96-133 fragment of bGH has activity, even though it is only 38 amino acids long, i.e., about 20% of the original protein. Clearly, the art could in fact expect to make substantial deletions in bGH, and still retain some activity.

Claim 29 has been amended to require that the GH antagonist comprise an amino acid sequence comprising residues corresponding to residues 96-133 of bovine growth hormone, which was identified as a growth-promoting fragment by the prior art cited at page 2, lines 20-35.

This amendment is supported not only by page 2, lines 20-35, but also by page 18, lines 2-3, and page 25, lines 27-32.

New claim 74 requires a polypeptide with four alpha helices; by implication, residues on the N-terminal side of the first helix or the C-terminal side of the fourth helix could be truncated off. The residue numbers are taken from page 15, lines 22-23, but are indicated to be approximate in view of page 15, lines 6-8 and 23-33, and page 18, line 9.

Prior claim 65 prohibits deletions (including truncations), but by the language "comprises" allows fusions at the N- or C-termini, see page 18, lines 10-12. Replacements (substitutions) are preferred to deletions according to page 18, lines 1-2.

2.2. To the extent that the rejection is applied to pure

substitution mutants of a vertebrate GH, because the substitutions could modify up to 50% of the reference GH, we believe that the Examiner has not rebutted applicants' evidence that so much of the protein could be modified with a reasonable expectation of success of retention of receptor binding activity.

In this regard, it must be observed that the standard looks at the guidance provided by the specification, and that the law does not require that all of such guidance be "hard wired" into the claim. See General Electric Co. v. United States, 206 USPQ 260, 283-4 (Ct. Claims Trial Div.).

The guidance provided by applicants included:

(1) the 3D structure of porcine GH, see page 15, lines 1-10;

(2) X-ray studies of bovine GH and human GH, see page 15, lines 8-10;

(3) the sequences of the vertebrate GHs, see page 15, lines 34-35, and inspection of those sequences for residues not conserved, see page 17, line 35 to page 18, line 2; page 16, lines 16-28;

(4) 3D structural models deduced for other GHs based on (1) and on the aligned primary and secondary structures, see page 15, lines 10-18;

(5) the identification of putative binding domains by homologue-scanning mutagenesis and alanine scanning mutagenesis, both already applied to hGH, see page 16, line 34 to page 17, line 15;

(6) the preference for substitutions that replace an amino acid with one of similar size and polarity, see page 23, lines 14-15;

(7) the preference for substitutions which occur more frequently between homologous proteins of different organisms, see page 25, lines 33-37, and in particular the conservative substitutions defined at page 26, lines 1-20;

(8) the preference for mutation at surface residues, other than the receptor binding site, over interior residues, these being distinguished by inspection of the known or predicted 3D structure, see page 25, lines 5-14;

(9) the preference for insertions or deletions at flexibly surface loops, see page 25, lines 14-16;

(10) the disclosure of combinatorial phage libraries for simultaneously exploring the effects of several different mutations, see page 22, line 6 to page 23, line 4, which indeed had already been done with GH (and which showed that the effects of mutations were ordinarily additive).

The data on pp. 9-10 of the last amendment, which is for fish GHs, shows that a polypeptide can have only 33-37% identity with human GH and still bind to a vertebrate GH receptor.

Thus, we have evidence of (1) extensive guidance as to where and how to mutate, and (2) that substantial mutations, even exceeding 50%, may be made in a vertebrate GH without loss of vertebrate GH receptor binding activity.

The burden of proof now shifts to the Examiner.

The only technique discussed by the Examiner was alanine scanning mutagenesis. At page 7, lines 6-10, the Examiner declared:

> Furthermore, alanine-scanning mutagenesis only mutates one amino acid at a time. It is well understood in the art that receptor binding is a coordinated effort of many amino acids throughout the protein. If amino acid position 12 is critical, the mutation of 11 or 13 may alter binding due to amino acid interaction or spacial arrangements that cannot be predicted by alanine-scanning mutagenesis.

Multiple mutation studies of many proteins have confirmed that <u>most</u> mutations are <u>reasonably</u> additive in their effects. See Wells, Biochemistry, 29:8509-17 (1990); Sandberg and Terwilliger, Proc. Nat. Acad. Sci. (USA), 90:8367-71 (1993);

Gregoret and Sauer, Proc. Nat. Acad. Sci. (USA), 90:4240-50 (1993); Schreiber and Fersht, J. Mol. Biol., 248:478-86 (1995); Lowman and Wells, J. Mol. Biol. 234:564-78 (1993); Lawman, et al., J. Biol. Chem., 266:10982-8 (1991); Lin, et al., Proc. Nat. Acad. Sci. (USA), 91:10265-9 (1994); Venkatachalam, et al., J. Biol. Chem., 269:23444-50 (1994); Akasako, et al., Biochemistry, 34:8115-22 (1995); Behravar, et al., Eur. J. Biochem., 198:589-92 (1991); Lin, et al., Proc. Nat. Acad. Sci. (USA), 91:10265-9 (1994); Zuckerman, et al., Proc. Nat. Acad. Sci. (USA), 89:4505-9 (1992).

According to Lowman et al., J. Biol. Chem., 266:10982-8 (1991), the HGH-receptor binding properties of human placental lactogen was improved about 500-fold by five simultaneous mutations, with "reasonably additive" effects.

Genentech, WO90/04788 discusses the HGH double mutants K168A/E174A, R18N/I179M, and K172A/F176A at page 99. It comments that the changes in free energy of binding to hGH or hPRL receptors is "strikingly additive"; details are given in Table XXI on page 100. Genentech comments that "such additivity oreates an extremely predictable situation for engineering variants of hGH". Similarly, in multiple mutation of hPRL to improve its binding to the hGH receptor, Genentech observed that the mutational effects were sufficiently additive to allow rational combination of single substitutions, see page 113, lines 11-20 and 29-32.

Additivity was also observed when mutations recommended by different combinatorial mutagenesis experiments were combined. Thus, Genentech WO97/11178 combined the three best hGH variants from its helix 1 library (positions 10, 14, 18, 21) with the three best from its helix-46 library (positions 167, 171, 174, 176, 179). Cumulative enhancements were observed, and the actual affinity was deemed "reasonably close" to the sum of the component affinities, see page 92, line 3.

At page 93, WO97/11178 notes that in a few instances, more complex additivity patterns are observed. However, one can avoid reliance on simple additivity by constructing combinatorial libraries with mutations directed to the residues shown previously to be of interest. Only multiple mutations with still favorable combinations will be selected.

In WO97/11178, mutations in positions 10, 14, 18 and 21 were combinatorially combined with mutations in positions 167, 171, 175 and 179, see pp. 93-96. The highest affinity mutant was 717B.1, which was almost identical to the BD variant found by nonrandom combination (3x3) of the best variants from the random helix=1 and random helix=4D libraries.

It is known in the art to conduct what is called binomial alanine mutagenesis. See Gregoret, et al., Proc. Nat. Acad. Sci. (USA), 90:4246-50 (1993). This is the combinatorial equivalent of the single substitution method disclosed by Cunningham. Each of n positions, where n>1, is allowed to be randomly either Ala or the wild-type residue. So a given molecule may have 0-n alanine substitutions.

At the time of filing, Ladner disclosed that a combinatorial phage display library could present 10^8-10^{10} different sequences. 10^8 is about 2^{27}, 10^{10} is about 2^{30}. So that implies that one could simultaneously test 28-30 positions. That is more than ample to detect coordinated mutation effects, indeed, sampling 2-3 positions at one time would find most if not all of them.

Even if a combination of favorable single substitutions is not simply additive, this is not necessarily a problem. The combination is undesirable only if the effect of the second mutation on the first is antagonistic (combination worse than either single substitution alone), not if it is neutral or still partially additive (let alone synergistic).

2.3. Applicants have pointed out that to the extent that their guidance might occasionally falter, i.e., point to an

inoperative species, such species are excluded by the "activity" limitation Ex parte Mark specifically approved this use of a "activity" limitation.

The Examiner attempts to distinguish Mark because there are only a few cysteines, in a given protein, which need to be tested. While that is true, the Mark claim was not limited to a single protein family. Rather, it covered cysteine-depleted muteins of any native protein. So one must multiply the average number of cysteines per protein by the number of proteins of interest.

In general, a claim is not invalid because it reads upon some inoperative embodiments, especially if those embodiments are unreasonable, see In re Skrivan, 166 USPQ 85 (CCPA 1970); In re Geerdes, 180 USPQ 789,793 (CCPA 1974); In re Cook, 169 USPQ 298, 302 (CCPA 1971); In re Myers, 161 USPQ 668, 672 (CCPA 1969), or relatively small in number, see Atlas Powder Co. v. E. I. duPontde Nemours & Co., 224 USPQ 409 (Fed. Cir. 1984); Hybritech, Inc. v. Abbott Laboratories, 4 USPQ2d 1001, 1012 (C.D. Calif. 1987), aff'd 7 USPQ2d 1191 (Fed. Cir. 1988).

While an "operability" limitation is not required by law, it certainly has the effect of preventing the coverage, however artificially, of inoperative embodiments, and hence is relevant to enablement.

2.4. With regard to relevance of the 13 prior patents which combined "percentage identity" (in three cases, 50% or less) and activity limitations, the Examiner refers to the "practice of the PTO" and the old Hutchison decision as the basis for ignoring them. The PTO is not the final arbiter of patent law and practice; all PTO decisions are appealable to the courts. It is clear from Andrew Corp. and Brian (last amend. p. 8) that prior patents are relevant and cannot be ignored.

Courts try to follow the precedent of prior decisions (stare decisis) because that leads to greater certainty among the public

as to what the law is. We would think that the public also should receive consistent examination of patent applications by the PTO. This is a point emphasized in both Andrew Corp. and Brian as justification. The Hutchison case did not look at a general pattern of practice, but only at a single prior patent.

While the Examiner says that the "practice of the PTO" is to ignore prior allowances, MPEP §706.04 calls for the exercise of "great care" in authorizing the rejection of a previously allowed claims and for giving "full faith and credit" to the search and action of a previous examiner "unless there is clear error".

35 USC §282 says that a patent is presumed valid; attacking a feature in a patent application claim which is duplicative of one in a patent claim as invalid is in effect a collateral attack on the validity of the patent, unless the Examiner points out why the two are distinguishable. In this regard, the Examiner's attention is directed to claim 26 of Kopchick, USP 5,681,809.

Respectfully submitted,

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